Virology **270,** 278–285 (2000)
doi:10.1006/viro.2000.0304, available online at http://www.idealibrary.com on IDEAL®

Varicella-Zoster Virus Infection of a Human CD4-Positive T-Cell Line

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Received February 1, 2000; returned to author for revision February 25, 2000; accepted March 6, 2000

Varicella-zoster virus (VZV) is a human α-herpesvirus that causes varicella (chickenpox) at primary infection and may reactivate as herpes zoster. VZV is a T-lymphotropic virus *in vivo.* To investigate the T-cell tropism of VZV, we constructed a recombinant virus expressing green fluorescent protein (VZV-GFP) under the CMV IE promoter. Coculture of VZV-GFP-infected fibroblasts with II-23 cells, a CD4-positive human T-cell hybridoma, resulted in transfer of virus to II-23 cells. II-23 cells are susceptible to VZV-GFP infection as demonstrated by expression of immediate/early (IE62), early (ORF4), and late (gE) genes. Recovery of infectious virus was limited, with only 1 to 3 in 10^6 cells releasing infectious virus by plaque assay, indicating that transfer of virus results in a limited productive infection. *In vitro* infection of II-23 cells will be useful for further analysis of VZV tropism for T-lymphocytes.

INTRODUCTION

Varicella-zoster virus (VZV) is a human α-herpesvirus that causes varicella (chickenpox) as the primary infection in susceptible individuals and may reactivate as herpes zoster (shingles) (Arvin, 1996). The α-herpesvirus subfamily of human *Herpesviridae* also includes herpes simplex virus (HSV)-1 and HSV-2. While most α-herpesviruses infect a variety of cell types in culture and a wide spectrum of species and tissue types *in vivo*, VZV infection is highly restrictive with regard to species and tissue type, propagating most efficiently in human cells, including most human diploid fibroblast cell lines (Arvin, 1996). VZV has also been grown in guinea pig embryo and monkey kidney cells, and less efficiently in EBV-transformed B-cells and neuronal cell lines (Assouline *et al.,* 1990; Fioretti *et al.,* 1973; Geder *et al.,* 1965; Koropchak *et al.,* 1989).

Our experience with the SCID-hu mouse model of VZV pathogenesis revealed that VZV possesses the ability to infect and replicate in T-cells within fetal thymus/liver (thy/liv) implants and is released by infected T-cells as cell-free virus (Moffat *et al.,* 1995). The lymphotropism of VZV is a characteristic shared with human herpes virus 6 (HHV-6) and HHV-7, and is an essential component of natural infection (Arvin, 1996; Secchiero *et al.,* 1994). Primary infection of VZV involves spread of infected peripheral blood mononuclear cells (PBMCs) to distant sites before effective VZV specific immunity is induced (Arvin, 1996). T-lymphocyte-mediated immune responses act to eliminate virus-infected PBMCs and to restrict virus replication to skin lesions. If the immune response is inadequate, VZV has the potential to cause disseminated infection of the lungs, liver, central nervous system, and other organs (Myers, 1979). VZV DNA has been recovered in 11–24% of PBMCs taken within 24 h of onset of the rash from healthy individuals with acute varicella by using cell culture methods and in 67–74% of samples tested by *in situ* hybridization or polymerase chain reaction (Koropchak *et al.,* 1989, 1991; Sawyer *et al.,* 1992). The PBMC subpopulations that harbor infectious virus during the cell-associated viremic phase have not been identified in the natural host because of the low frequency of positive cells (Asano *et al.,* 1985; Koropchak *et al.,* 1989; Mainka *et al.,* 1998). VZV replicates in both CD4+ and CD8+ T-lymphocytes in the SCID-hu thy/liv model (Moffat *et al.,* 1995).

The efficiency of infection of lymphocytes *in vitro*, using either PBMCs or umbilical cord mononuclear cells is low (1–5%) and VZV-positive cells are difficult to identify by specific phenotype and separate from the infected fibroblast inoculum (Koropchak *et al.,* 1989; Soong *et al.,* 1999). Despite extensive testing of CD4+ Jurkat and lymphoblastoid-derived T-cell lines maintained with various growth factors, VZV T-cell tropism was not observed *in vitro* in our experience until the CD4+ T-cell hybridoma, II-23 cell line, was evaluated. The II-23 T-cell hybridoma, which was constructed by fusion of lectin-activated peripheral blood lymphocytes and CEM.TET1, a variant of the CEM lymphoblastoid cell line, was initially characterized by Ware *et al.* (1986) as an inducible model system for the production of growth inhibitory/cytolytic and growth promoting lymphokines and cytotoxic T-cell

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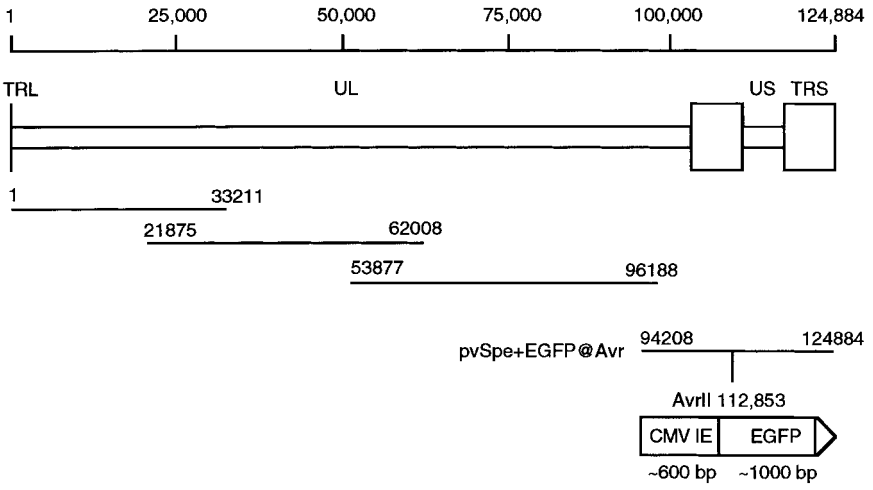

FIG. 1. Design of VZV-GFP insertion cassette. Four overlapping fragments of genomic VZV DNA were cotransfected into melanoma cells. Cosmid pvSpe+EGFP@Avr (spanning VZV nucleotides 94208 to 124884) contains a 1.6-kb EGFP cassette containing the CMV IE promoter, the EGFP coding region, and the SV40 poly A region. The cassette was ligated into a unique *Avr*II site at VZV nucleotide 112853.

function. Cellular entry mechanisms for herpes simplex virus have been identified using the II-23 cell line (Mauri *et al.,* 1998).

Herpesvirus recombinants expressing the green fluorescent protein (GFP) from the bioluminescent jellyfish *Aequorea victoria* have proved useful in the study of viral entry, protein trafficking, and pathogenesis. Several studies have reported using GFP as a marker for viral entry or fusion with herpesviral structural proteins to visualize trafficking of virion components in live, infected cells (Desai and Person, 1998; Elliott and O'Hare, 1999; Foster *et al.,* 1998). We constructed a recombinant VZV (Oka strain) expressing green fluorescent protein (VZV-GFP) under the CMV immediate/early (IE) promoter. Using GFP expression as a marker for viral entry, we demonstrated that cultured II-23 T-cells are susceptible to VZV infection. This novel model of T-cell infection with VZV should be useful for the systematic analysis of host cell surface components that mediate T-cell infection as well as other studies of VZV pathogenesis.

RESULTS

Generation of VZV-GFP

The VZV genome is a linear double-stranded DNA molecule consisting of approximately 125,000 bp that, when cloned in cosmid vectors and transfected into susceptible cells, results in the generation of mature infectious virions (Cohen and Seidel, 1993; Dumas *et al.,* 1981; Mallory *et al.,* 1997). We used a cosmid system for generation of recombinant VZV-expressing green fluorescent protein. In this system, four overlapping fragments of genomic DNA from the vaccine Oka strain of VZV were ligated into SuperCos1 vectors (Stratagene, La Jolla, CA); these cosmids were kindly provided by George Kemble (Aviron, Inc., Mountain View, CA). The cosmid clone pvSpe21+EGFP@Avr, consisting of a 1.6-kb EGFP (enhanced green fluorescent protein) cassette containing the CMV IE promoter, the EGFP coding region, and the SV40 poly A region amplified from the vector pEGFP-C1 (Clontech Inc.), was ligated into a unique *Avr*II site in cosmid pvSpe21 at VZV nucleotide 112853 (Fig. 1).

Transfection of VZV cosmids

Purified DNA from intact cosmids and pvSpe21+EGFP@Avr cosmid were cotransfected into human melanoma cells (Mallory *et al.,* 1997). Cytopathic effect was evident 5 to 6 days after transfection. Green fluorescence denoting GFP protein expression was associated with infectious foci. GFP expression was localized to the cytoplasm of virally infected cells and could be detected by fluorescence microscopy and FACS analysis early in infection. The resulting recombinant VZV-expressing GFP (VZV-GFP) was transferred from melanoma cells into human embryonic lung fibroblast (HELF) cells by inoculation of uninfected HELF cells. GFP expression in HELF cells was stable at low passage and distributed evenly in the cytoplasm of infected cells. Growth kinetics and viral titers of VZV-GFP were similar to vaccine Oka in fibroblasts. In Fig. 2A, a VZV-GFP-infected HELF cell monolayer exhibited typical early VZV plaque morphology. GFP expression was observed in the same plaque, limited to the area of the syncytia, and was evenly distributed in the cytoplasm of rounded, virally infected cells (Fig. 2B).

VZV-infection of II-23 cells

The strong association of progeny virions with the infected cell results in low titers of cell-free VZV obtained by conventional methods of virus preparation. We inoc-

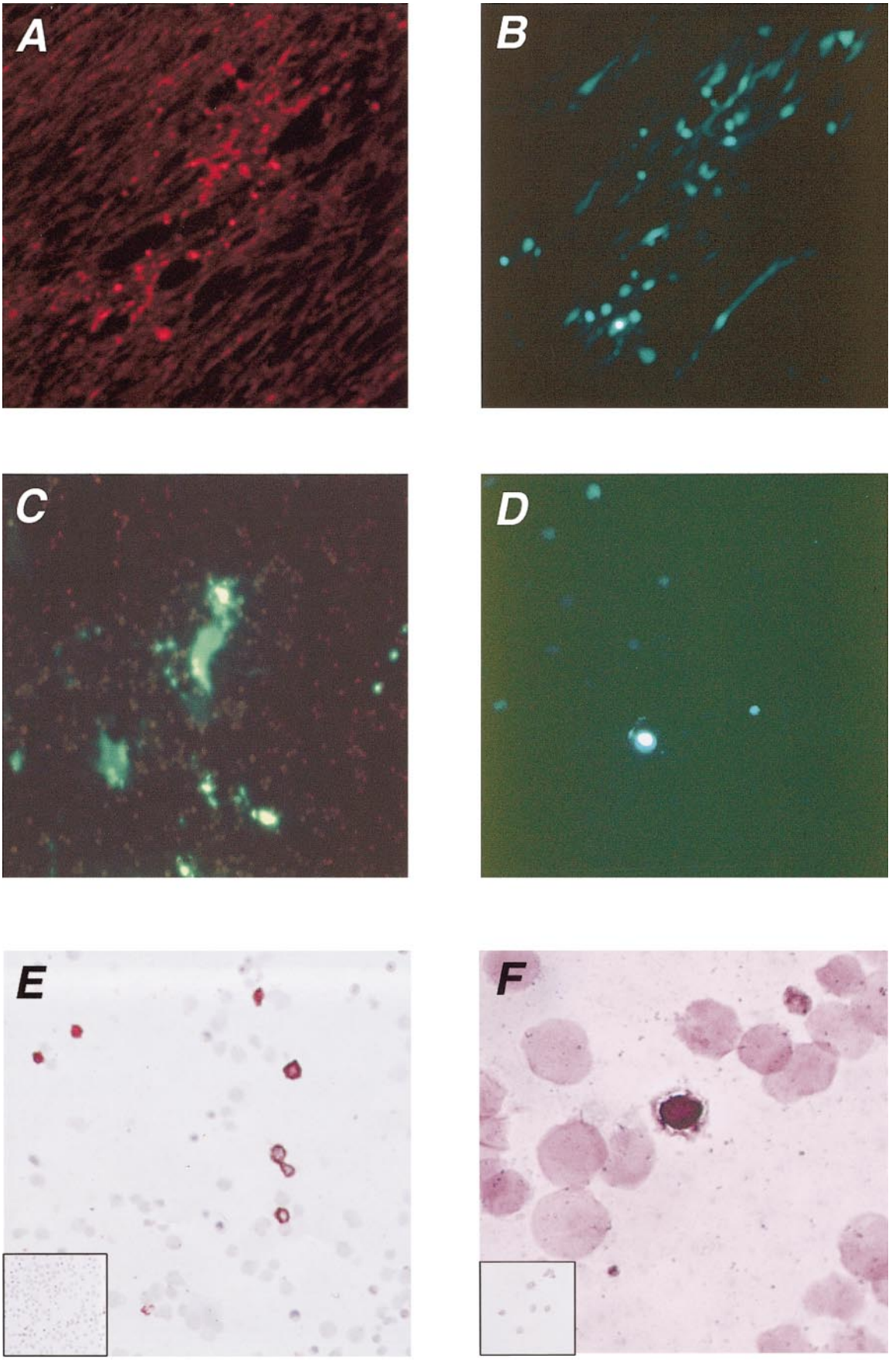
A
B
C
D
E
F

TABLE 1

Efficiency of VZV-GFP transfer to II-23 cells

IL-23 cell density	Inoculum ratio (VZV-GFP infected:uninfected HELF cells)			
	1:1	1:10	1:100	1:1000
10^7/ml	8.3 ± 3.3[a]	3.0 ± 0.1	0.5 ± 0.3	0.2 ± 0.1
10^6/ml	8.7 ± 1.4	10.1 ± 1.1	21.8 ± 2.1	10.1 ± 1.4
10^5/ml	10.6 ± 2.6	14.6 ± 0.5	30.8 ± 1.2	14.9 ± 1.7

[a] Percentage CD_4/CD_{45}/GFP-positive cells ± SE.

ulated II-23 cells by coculture with a VZV-GFP-infected HELF cell monolayer (Fig. 2C) at 2–3+ cytopathic effect. Using a 1:100 starting ratio of infected to uninfected HELF cells as the cell-associated VZV-GFP inoculum, maximal transfer of virus was achieved by Day 3 of coculture, resulting in up to 30% of II-23 cells expressing GFP (Table 1). At equivalent CPE, low density cultures resulted in significantly increased viral transfer (30.8% at 10^5 cells/ml compared with 0.5% at 10^7 cells/ml). GFP expression in II-23 cells was detected after 24 h in coculture and peaked at Day 3 (Fig. 3). Coculture of II-23 cells beyond 3 days resulted in a significant degradation of the integrity of the VZV-GFP HELF cell monolayer and did not increase levels of viral entry. As expected, transfer of virus to II-23 cells was not synchronous, resulting in a range in the intensity of GFP expression (Fig. 2D). Activation of II-23 cells with a combination of PMA (protein kinase C pathway) and ionomycin (ionophore effect) may upregulate expression of certain herpesvirus entry mediators (Mauri *et al.,* 1998). Prior activation of II-23 cells with PMA ± ionomycin did not increase susceptibility of II-23 cells to VZV-GFP compared with that of untreated controls.

To verify that GFP expression was the result of viral entry and not endocytosis of soluble GFP or fusion with VZV-GFP-infected HELF cells, II-23 cellular transcription was inhibited with actinomycin D prior to coculture. II-23 cells incubated with 0.1 μg/ml actinomycin D expressed significantly lower levels of GFP than untreated II-23 cells (3.2 compared with 8.0%) after 24 h of coculture. The reduction in GFP expression following actinomycin D treatment indicates that viral gene transcription is required for GFP expression.

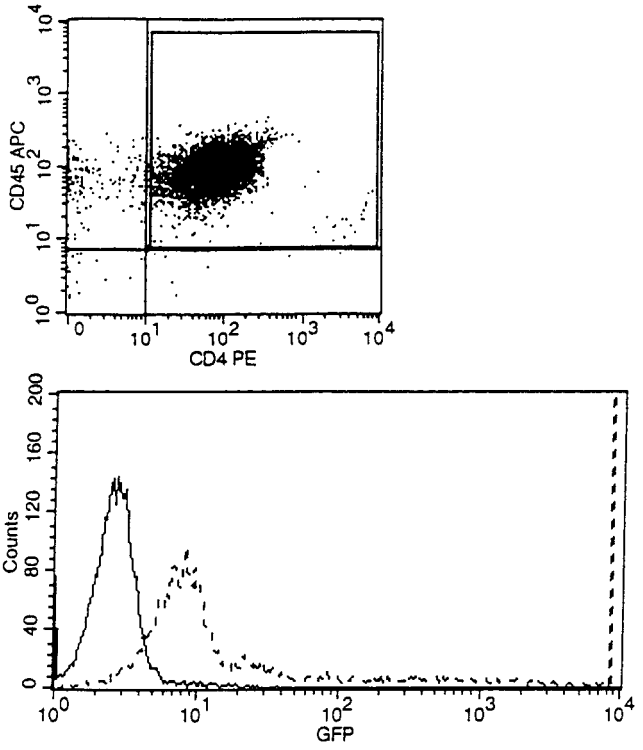

FIG. 3. Flow cytometric analysis of VZV infection of II-23 cells. VZV-GFP-infected II-23 cells were decanted from coculture with VZV-GFP-infected HELF cells and labeled with mouse monoclonal anti-CD4-phycoerythrin (PE) and anti-CD45-allophycocyanin (APC) for fluorescent-activated cell sorting. Top panel: >97% of cells stained were CD4 and CD45 positive; bottom panel: GFP expression in CD4/CD45 II-23 cells. Uninfected II-23 cells (no coculture, control) are represented by the solid histogram. VZV-GFP-infected II-23 cells are represented by the hatched histogram. In this representative sample, of the CD4/CD45 II-23 cells gated for FACS analysis, 16% are GFP positive.

VZV-infected II-23 cells are not productively infected

Plaque assay of VZV-infected II-23 showed recovery of infectious virus from 1 to 3 in 10^6 II-23 cells. The low level of infectious virus suggests that, while II-23 cells are susceptible to viral entry and some gene expression, the host cell does not provide a suitable environment for efficient replication. No infectious virions were recovered when VZV-infected II-23 cells were treated by sonic disruption prior to titration.

Characterization of VZV-GFP infection of II-23 cells

Western blot analysis. Western blot analysis was performed to see if the limited productive infection resulted

FIG. 2. Histological characterization of VZV-infected II-23 cells. (A) Propidium iodide (PI) stain of a VZV-GFP plaque on a HELF cell monolayer (magnification, 200×). (B) GFP expression in a VZV-GFP plaque on a HELF cell monolayer, same plaque as (A) (magnification, 200×). (C) II-23 cells are susceptible to infection with VZV-GFP by coculture on VZV-infected HELF cell monolayers, dual PI/GFP stain of II-23 cells on a HELF cell monolayer infected with VZV-GFP (magnification, 200×). (D) FACS-sorted GFP-positive II-23 cells Day 3 postinfection with VZV-GFP (magnification, 200×). (E) Immunohistochemical staining of VZV protein in II-23 cells infected with VZV-GFP using the IgG fraction of a high titer polyclonal serum, lower left-hand panel inset = staining with nonimmune IgG (magnification, 200×). (F) *In situ* hybridization showing VZV DNA integration in FACS-sorted II-23 cells, lower left-hand panel inset = negative control (magnification, 400×).

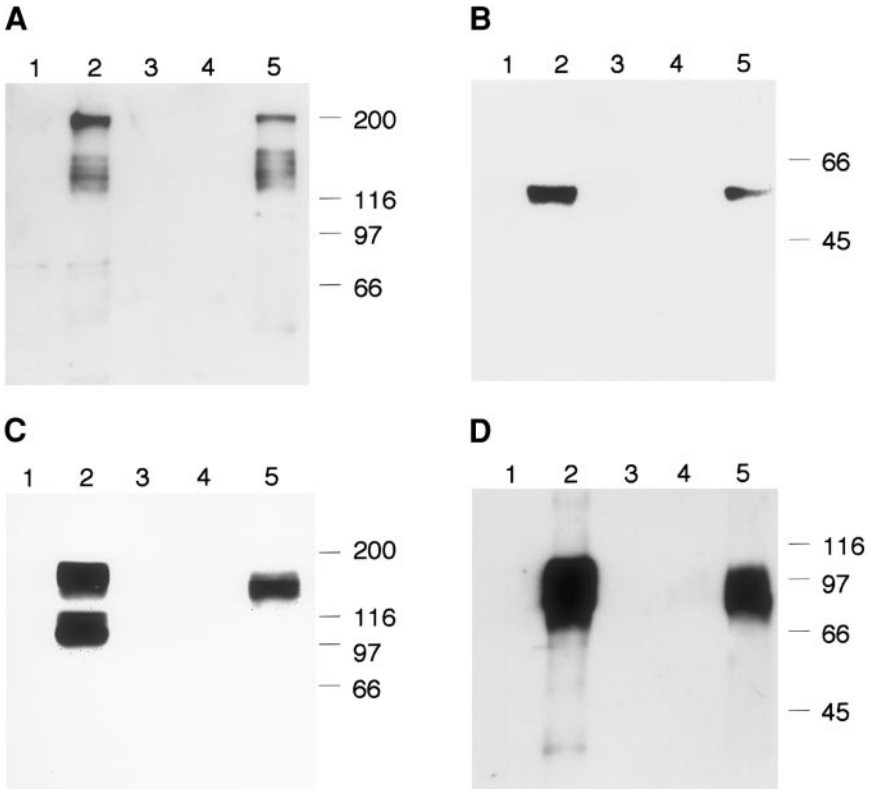

FIG. 4. Western blot analysis of VZV protein expression in II-23 cells. Western blot for VZV protein expression in uninfected HELF cells (lane 1), VZV-GFP-infected HELF cells (lane 2), uninfected II-23 cells (lane 3), FACS-sorted CD4+/CD45+/GFP-negative II-23 cells (lane 4), and FACS-sorted CD4+/CD45+/GFP-positive II-23 cells (lane 5) using the following primary antibodies: α-IE62 gene product (A), α-ORF4 gene product (B), IgG fraction of a high titer polyclonal serum (C), mAb to VZV glycoprotein E.

from a block in the expression of immediate early, early, or late genes. VZV-infected II-23 cells express abundant VZV protein of α, β, and γ subclasses. The immediate/early gene IE62, a major component of the viral tegument, was expressed in VZV-infected II-23 cells (Fig. 4A). Monoclonal antibody to IE62 recognizes several phosphoproteins in VZV-infected cells between 175 and 180 kDa. ORF4, a 51-kDa protein with sequence homology to HSV-1 ICP27, is a putative early gene and was expressed in VZV-infected II-23 cells (Fig. 4B). VZV proteins of the γ subclass were detected using the IgG fraction of a high-titer polyclonal human serum, which recognizes predominantly late viral glycoproteins and antibody to glycoprotein E, the most abundant VZV glycoprotein made in virus-infected cells (Figs. 4C and 4D).

Immunohistochemical staining. The distribution of viral protein expression and viral DNA in VZV-infected II-23 cells was evaluated by immunohistochemical staining and *in situ* hybridization. Viral protein expression, while present in only a fraction of cells expressing GFP, was apparent in an even distribution on the surface of II-23 cells (Fig. 2E). GFP expression was more sensitive than viral DNA for detecting infected cells (Fig. 2F). Controls using nonimmune serum for immunohistochemical stains and using the vector alone as a probe for *in situ* hybridization were negative (Figs. 2E and 2F: inset, lower left). The polyclonal antibody used for immunohistochemistry recognizes predominantly late glycoproteins.

DISCUSSION

The specific targeting of cells of the immune system is a characteristic of herpesviruses, many of which infect T-cells, B-cells, or monocytes during acute or persistent infection. Using GFP expression by a VZV-GFP recombinant as a marker for viral entry, we demonstrated that II-23 cells are susceptible to infection with VZV. However, little infectious virus was released from GFP-expressing II-23 cells, suggesting that while II-23 cells are susceptible to viral entry, the host cell does not provide a suitable environment for productive infection. The extreme cell-associated replication of VZV precludes a precise analysis of its replication kinetics using a cell-free virus inoculum. By analogy with HSV, VZV is presumed to follow the cascade of immediate/early (α), early (β), and late (γ) gene transcription that characterizes HSV infection (Roizman, 1996). VZV proteins of α, β, and γ subclasses were expressed in II-23 cells. Expression of envelope glycoproteins on the cell surface was preserved and GFP expression could be detected in culture for several days postinfection.

A prior study of VZV T-cell tropism in our laboratory

showed that VZV entered PHA-activated T-lymphocytes but viral replication *in vitro* was impaired (Koropchak *et al.,* 1989). Soong *et al.* (1999) showed that 3–4% of cord blood T-cells, many of which express activation markers, were infected after coculture with VZV-infected fibroblasts. Productive infection follows virion entry only if intracellular conditions permit viral uncoating, transport to the nucleus, viral gene expression, replication, assembly, and egress. Perera *et al.* (1992) demonstrated that the ORF62 gene product, the immediate/early viral transactivator, was capable of upregulating the expression of VZV proteins of α, β, and γ subclasses in activated T-lymphocytes, using an *in vitro* transient expression assay (Perera *et al.,* 1992). These experiments suggested that T-cells possess the machinery required for viral transactivation and expression of immediate/early, early, and late gene products. VZV infection of II-23 cells, like cord blood T-cells, was associated with synthesis of all classes of viral proteins. While no mechanism has been identified for the limited replication of VZV during T-cell infection, VZV virions may enter most lymphocytes by a pathway that does not support productive infection, virion assembly, or egress is blocked (Koropchak *et al.,* 1989).

VZV spreads in cultured human diploid fibroblast monolayers in a cell-to-cell fashion, mediated by envelope glycoproteins that enable cells to fuse into megapolykaryocytes and form syncytia (Arvin, 1996; Grose, 1990). Similarly, polykaryocytes and syncytia that form in VZV-infected monolayers and the giant cells that form in VZV skin lesions are not observed when VZV infects T-cells in thy/liv implants (Moffat *et al.,* 1995). Unlike infection of tissue culture monolayers, in which infected cells fuse into multinucleated syncytia, histological analysis of GFP-expressing II-23 cells did not reveal polynucleated or fused cells, indicating a difference in pathogenic effect of VZV on T-cells.

Glycoproteins and other viral genes can affect VZV replication differentially in tissue culture cells and also T-cells and skin tissue in the SCID-hu model (Moffat *et al.,* 1995). The glycoprotein C of VZV was an essential virulence determinant for skin but not in T-cells in the SCID-hu model (Moffat *et al.,* 1998). The ORF47 protein kinase of VZV, which is dispensable for growth in human diploid fibroblasts *in vitro*, was demonstrated to be a determinant of T-cell tropism using the SCID-hu thy/liv model (Moffat *et al.,* 1998). II-23 cells provide another tool for the analysis of VZV gene products that mediate interaction with T-cells.

HSV-1 and -2 infect II-23 T-cells and dendritic cells by attachment of HSV envelope glycoprotein D (gD) to the cellular herpesvirus entry mediator HveA (Montgomery *et al.,* 1996; Salio, 1999). Since VZV does not possess a homolog to HSV gD, it may employ means of viral entry and egress not shared among other α-herpesvirus family members. Activation of II-23 cells results in transient expression of several TNF-related ligands on the cell surface (Ware *et al.,* 1992). However, activation with PMA and ionomycin did not alter susceptibility of II-23 cells to VZV infection, suggesting that proteins of this family are not indicated in VZV entry. Nevertheless, our results suggest that, while II-23 cells allow for VZV viral entry and protein expression, levels of VZV gene expression and DNA replication are not adequate for productive infection. The abortive replication cycle of VZV in II-23 cells should permit studies of viral entry and gene expression in VZV-infected T-cells.

MATERIALS AND METHODS

Generation of Oka strain of VZV-expressing GFP (VZV-GFP)

Four overlapping fragments of genomic DNA from the Oka strain of VZV were ligated into SuperCos1 vectors (Stratagene); these cosmids were kindly provided by George Kemble (Aviron, Inc.). The deletion of an *Avr*II site from the original cosmid vector at SuperCos1 nucleotide 3359 produced a unique *Avr*II site at VZV nucleotide 112853, in cosmid pvSpe21 (Fig. 1).

The cosmid clone pvSpe21+EGFP@Avr was generated as follows: A 1.6-kb EGFP (enhanced green fluorescent protein) cassette containing the CMV IE promoter, the EGFP coding region, and the SV40 poly A region was amplified from the vector pEGFP-C1 (Clontech Inc.) using PCR primers that annealed upstream of the CMV IE promoter and downstream of the SV40 polyA signal. The primers were designed so that they would introduce an *Avr*II restriction enzyme site at each end of the cassette. The PCR product was isolated and digested with *Avr*II. The cosmid vector pvSpe21 was digested at the unique *Avr*II site at nucleotide 112853, between ORF65 and 66. The EGFP cassette was ligated into the *Avr*II-cut cosmid vector and clones were isolated that contained the EGFP cassette in both orientations.

Cosmid transfections were done as previously described (Mallory *et al.,* 1997). After transfection, the melanoma cells were kept at 37°C for 3 to 4 days, trypsinized, and transferred to a 75-cm^2 flask; plaques appeared 5 to 6 days after transfection. Green fluorescence denoting GFP protein expression was observed by fluorescent microscopy and associated with infectious foci.

Viral infection and culture conditions

VZV-GFP was transferred from melanoma cells into human embryonic lung fibroblasts (HELF) by inoculation of uninfected HELF cells. Low-passage viral stocks were prepared and stored at −70°C in freezing media with 10% DMSO (Moffat *et al.,* 1995). The II-23 cell line (D7 subclone) was maintained in RPMI medium 1640 plus supplements and 10% fetal calf serum (FCS) (Ware *et al.,*

1986). Infection of II-23 cells is achieved by coculture of T-cells at 10^5–10^6 cells/ml on a monolayer of VZV-GFP-infected HELF cells at 2–3+ cytopathic effect (CPE) for 2–3 days with daily media changes. At high CPE, the infected HELF cell monolayer has poor integrity, resulting in inefficient transfer of VZV. The loss of monolayer integrity results in a decrease in II-23 to HELF cell contact and thus a decrease in viral transfer. At high target cell density (10^7/ml), cell viability was poor as a result of nutrient depletion.

To evaluate the susceptibility of activated II-23 cells to VZV entry, II-23 cells were activated for 4 h at 37°C with phorbol ester (100 ng/ml) or PMA (100 ng/ml) with ionomycin (1 μg/ml) prior to infection with VZV-GFP. To evaluate the ability of actinomycin D to abolish expression of GFP in II-23 cells, II-23 T-cell hybridomas were pretreated for 30 min with 0.1 μg/mL actinomycin D and then rested at 37°C for 6 h to allow for protein synthesis from preexisting mRNA. Cells were then infected with VZV-GFP as previously described. FACS analysis was performed, comparing the percentage of GFP-positive II-23 cells in actinomycin D-treated cells and controls.

FACS analysis and cell sorting

II-23 cells were removed from the VZV-GFP HELF cell monolayer by gentle pipetting, washed with phosphate-buffered saline (PBS), and counted. Cells were resuspended at a concentration of 10^7 cells/ml in PBS with 1% FCS. The cells were labeled with mouse monoclonal anti-CD4-phycoerythrin (PE) and anti-CD45-allophycocyanin (APC) (both from Becton Dickinson, Mountain View, CA) for 20 min at 4°C. The cells were then analyzed using a FACS Calibur or sorted using a FACS Vantage (Becton Dickinson, San Jose, CA). Three-color analysis permitted FACS sorting of GFP-positive and GFP-negative II-23 cells from cell-associated inoculum and cell debris (Fig. 3).

Infectious foci assay

FACS-sorted GFP-positive II-23 cells T-cells at 10^7/ml were serially titrated in 24-well dishes in triplicate, preseeded with 1.5×10^5 African green monkey kidney (Vero) cells. The dishes were incubated for 10 days at 37°C in 5% CO_2 and then fixed with 10% formalin. Green plaques were counted using an inverted fluorescent microscope. The number of infectious foci per well were calculated. Cell-free viral release was also evaluated by transwell assay. FACS-sorted GFP-positive cells were either (1) untreated, (2) sonicated for 10 s in tissue culture media, or (3) sonicated for 10 s in PSGC (85% PBS, 5% sucrose, 10% FCS, 0.1% Na-glutamate) media and laid onto a 3.0-μm-pore-size membrane over a Vero cell monolayer. As a control, FACS-sorted GFP-negative cells were treated in a similar manner. No infectious foci were counted above background levels.

Western blot

An aliquot of 10^6 FACS-sorted GFP-positive II-23 cells, GFP-negative II-23 cells, uninfected II-23 cells, uninfected HELF, and VZV-infected HELF cell controls were treated with detergent extract buffer containing protease inhibitors, sonicated for 1 min, and subjected to three freeze/thaw cycles. Following standard techniques, cell lysates were separated by SDS–PAGE and transferred to Immobilon-P PVDF membranes (Moffat *et al.,* 1995). The total amount of protein of each sample was equivalent, as verified by amido black stain. VZV proteins were detected with a high-titer polyclonal human immune serum and a secondary goat anti-human IgG horseradish peroxidase conjugate. Rabbit polyconal anti-IE62 and anti-ORF4 antibodies and a mouse monoclonal antibody to gE (kindly provided by Paul Kinchington and Charles Grose) were also used. ECL (enhanced chemiluminescence) reagents were added (Amersham, Buckinghamshire, UK).

Immunohistochemistry and *in situ* hybridization

FACS-sorted GFP-positive and -negative II-23 cells were cytospun onto poly-L-lysine-coated slides (EM Sciences, Ft. Washington, PA), fixed in 4% paraformaldehyde for 30 min at room temperature, washed in PBS, and air-dried overnight. Immunohistochemistry and *in situ* hybridization was done as described previously (Moffat *et al.,* 1995). The VZV probe consisted of a 12.9-kb biotinylated plasmid pVZV-C, that is a pBR322 vector carrying the *Hin*dIII fragment C of VZV genomic DNA. A negative control probe consisting of pBR322 vector alone was used at the same concentration.

ACKNOWLEDGMENTS

A.M.A. is the Lucile Salter Packard Professor of Pediatrics; this work was supported by NIH Grants AI20459 (A.M.A.) and AI33068 and CA69381 (C.F.W.). We acknowledge the expert technical assistance of Patricia Lovelace with fluorescence-activated cell sorting analysis.

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